Full Paper

Synthesis, X-ray Structure, and Pharmacological Activity of Some 6,6-Disubstituted Chromeno[4,3-*b*]- and Chromeno-[3,4-*c*]-quinolines

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Some chromeno[4,3-*b*]quinolines **4a–i** were obtained from β-chloro carboxyaldehydes **3a–c** with different aniline derivatives namely, aniline, 4-fluoroaniline, and 2-aminophenol. Surprisingly, **3a–c** reacted with 2-aminothiophenol and afforded the chromeno[3,4-*c*]quinoline derivatives **5a–c**. Single-crystal X-ray diffraction studies of **4e** and **5b** provided good support for the established structure. Compounds **4b** and **5b** showed significant anti-inflammatory and ulcerogenic score activities compared to that of indomethacin.

Keywords: Anti-inflammatory / β-Chloro carboxyaldehyde / Chromeno[4,3-*b*]quinoline / Chromeno[3,4-*c*]quinoline / X-ray structure determination

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Supporting Information for this article:
Crystallographic data for the structures of compounds **4e** and **5b** have been deposited at the CCDC as supplementary data, deposition no. CCDC No. 618025 and CCDC No. 618026 copies of the data can be obtained, free of charges on application to CCDC-12 Union Road, Cambridge CB2 1EZ, UK. E-mail: deposit@ccdc.cam.ac.uk.

Introduction

The Vilsmeier–Haack reaction of active methylene compounds mainly lead to the formation of β-halo carboxyaldehyde which constitute a class of compounds that have served as useful intermediates towards construction of different heterocyclic compounds [1]. Accordingly, some of β-chloro carboxyaldehydes were reacted with phenylhydrazine and 2-mercapto acetic acid and its ethyl ester to give the corresponding pyrazolo and thieno derivatives [2–6]. Some fluorobenzo[*c*]acridine derivatives were synthesized from the corresponding β-chloro carboxyaldehyde with fluoroaniline [7]. Acridine derivatives exhibit antitumor [8, 9], fungicidal [10, 11], antiparasitic [12], antimicrobial [13, 14], anti-inflammatory, and analgesic activities [15–17]. Some of the acridine derivatives also possess kinase inhibition properties [18]. Some quinoline derivatives showed strong activity, as HIV-integrase inhibitors [19–21] and also possessed interesting antifungal and herbicidal activity [22]. Moreover, benzopyran-4-ones constitute an important class of naturally occurring substances [23–25] and draw the attention of many researchers due to their well known properties as anti-tuberculosis [26] agents. Therefore, it seemed very interesting to investigate the chemistry of some hindered β-chloro carboxyaldehydes towards some aniline derivatives, namely aniline, 4-fluoroaniline, 2-aminophenol, and 2-aminothiophenol and to evaluate the pharmacological activity of some new products as anti-inflammatory and ulcerogenic score activities.

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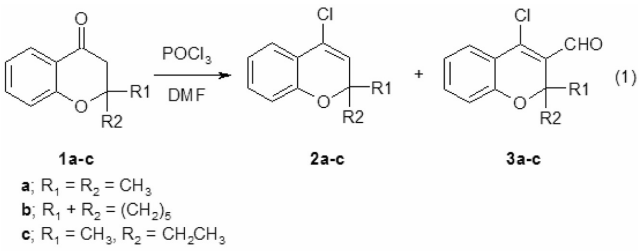


Scheme 1. Synthesis route of compounds **1 – 3.**

Results and discussion

Chemistry

Herein, we report the reaction of 4-chloro-2,2-disubstituted chromene-3-carboxyaldehyde with aniline, 4-fluoroaniline, 2-aminophenol, and 2-aminothiophenol to get some new chromenoquinoline derivatives. Chromanone derivatives **1a – c** were reacted with Vilsmeier reagent to afford the corresponding chloro derivatives **2a – c** along with β-chloro carboxyaldehyde derivatives **3a – c** (Scheme 1; [6]). *R,S*-4-Chloro-2-ethyl-2-methy(2*H*)chromene **2c** and *R,S*-4-Chloro-2-ethyl-2-methyl(2*H*)chromene-3-carboxyaldehyde **3c** have not been described before and they are established by physical and spectral data (Tables 1, 2).

Ray *et al*. have reported the reaction of 1-chloro-3,4-dihydro-2-naphthaldehyde with 2- and 4-fluoroaniline in ethanolic HCl (catalytic) to get the corresponding arylimine hydrochlorides [7] which were heated at 200-250°C to give 9-fluoro- and 11-fluoro-3,4-dihydrobenzo[*c*]acridine, respectively. Therefore, 4-chloro-2,2-disubstituted(2*H*)chromene-3-carboxyaldehydes **3a-c**, reacted with aniline in acetic acid under reflux for 3 h, afforded the corresponding products (6*H*)chromeno[4,3-*b*]quinolines **4a – c** rather than (6*H*)chromeno[3,4-*c*]quinolines **5a – c** (Scheme 1).

The chemical structures of compounds **4a – c** were established by physical and spectral data as well as elemental analyses (Tables 1, 2). The mass spectrum of **4a** shows the prominent ion peak M^+ at *m/z* 261 (20%). Accordingly, 4-chloro-2,2-disubstituted(2*H*)chromene-3-carboxyaldehydes **3a – c** reacted with 4-fluoroaniline in acetic acid under reflux for 3 h and afforded the corresponding products 9-fluoro(6*H*)chromeno[4,3-*b*]quinolines **4d – f** rather than 11-fluoro(6*H*)chromeno[3,4-*c*]quinolines **5d-f** (Scheme 1). The spectral data (IR, ^{1}H- and ^{13}C-NMR, and MS) as well as elemental analyses established the structures of **4d – f**. The ^{13}C-NMR of **4e** is in accordance with that of 9-fluoro- and 11-fluoro-3,4-dihydrobenz[*c*]acridine [7] (Table 2). The mass spectrum of **4e**, for example, shows the prominent ion peak at *m/z* 319 M^+ (34%). Moreover, the single crystal X-ray of **4e** gives a good evidence of the formation of the regioisomers **4a – i** rather than **5a – i**. The single crystal X-ray determination of **4e** is shown in Fig. 1.

On the other hand, 4-chloro-2,2-disubstituted(2*H*)chromene-3-carboxyaldehydes **3a – c**, reacted with 2-aminophenol, afforded the corresponding products 11-hydroxy (6*H*)chromeno[4,3-*b*]quinolines **4g – i** (Scheme 1). The structures of **4g – i** were established by spectral data

Table 1. Physicochemical data of the new compounds.

Compd.	Mp. (°C)	Yield (%)	Mol. formula Mol. Wt.
2c	Colorless oil	40	$C_{12}H_{13}ClO$ 208.67
3c	Yellow oil	55	$C_{13}H_{13}ClO_2$ 236.68
4a	Yellow/104 – 106	30	$C_{18}H_{15}NO$ 261.33
4b	Yellow/139 – 140	19	$C_{21}H_{19}NO$ 301.39
4c	Yellow /129 – 130	57	$C_{19}H_{17}NO$ 275.35
4d	Yellow/130 – 131	38	$C_{18}H_{14}FNO$ 279.32
4e	Yellow/179 – 180	19	$C_{21}H_{18}FNO$ 319.38
4f	Yellow/158 – 159	18	$C_{19}H_{16}FNO$ 293.34
4g	Yellow/57 – 58	22	$C_{18}H_{15}NO_2$ 277.33
4h	Yellow/114 – 115	14	$C_{21}H_{19}NO_2$ 317.36
4i	Yellow/145 – 146	14	$C_{19}H_{17}NO_2$ 291.35
5a	Yellow/120 – 121	13	$C_{18}H_{15}NO$ 261.33
5b	Yellow/192 – 193	55	$C_{21}H_{19}NO$ 301.39
5c	Brown oil	43	$C_{19}H_{17}NO$ 275.35
7	Yellow/226 – 227	71	$C_{21}H_{20}ClNOS$ 369.88

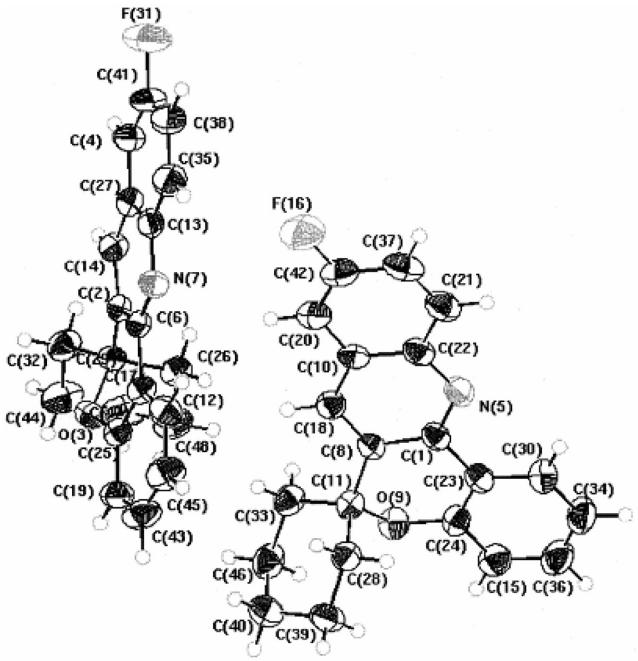


Figure 1. Molecular structure of compound **4e;** ellipsoids of thermal vibration are shown with 50% probability.

Table 2. IR, ^{1}H-, ^{13}C-NMR, and mass spectra for the new compounds.

Compd.	IR (cm^{-1})	^{1}H- and ^{13}C-NMR (δ ppm)	MS m/z (%)
2c	2972, 2929,1631, 1605, 1480, 1453, 1249, 752	1.02 (t, *J* = 7.3 Hz, 3H, CH_3CH_2), 1.44 (s, 3H, CH_3), 1.78 (q, *J* = 7.3 Hz, 2H, CH_3CH_2), 5.75 (s, 1H, 3-H), 6.84 (d, *J* = 8.2 Hz, 1H, ArH), 6.91-6.99 (m, 1H, ArH), 7.18–7.29 (m, 1H, ArH), 7.47 (dd, *J* = 7.4 Hz, 1.2 Hz, 1H, ArH).	
3c	2970, 2932, 1673, 1600, 1559, 1454, 1293, 756	0.92 (t, *J* = 7.4 Hz, 3H, CH_3CH_2), 1.60 (s, 3H, CH_3), 1.70–1.88 (m, 1H, $CH_3CH_aH_b$), 2.23-2.41 (m, 1H, $CH_3CH_aH_b$), 6.85 (d, *J* = 8.2 Hz, 1H, ArH), 6.97–7.04 (m, 1H, ArH), 7.21–7.42 (m, 1H, ArH), 7.70 (d, *J* = 7.8 Hz, 1H, ArH), 10.29 (s, 1H, CHO).	
4a	2959, 2925, 1728, 1602, 1459, 1263	1.78 (s, 6H, 2 CH_3), 6.99 (d, *J* = 8.1 Hz, 1H, ArH), 7.14–7.17 (m, 1H, ArH), 7.35–7.38 (m, 1H, ArH), 7.49–7.51 (m, 1H, ArH), 7.67–7.80 (m, 1H, ArH), 7.92 (s, 1H, ArH), 8.13 (d, *J* = 8.4 Hz, 1H, ArH), 8.50–8.53 (m, 1H, ArH), 7.78 (d, *J* = 8.1 Hz, 1H, ArH).	261 (M, 20), 245 (M − CH_3, 100), 230 (2), 217 (16), 149 (8), 123 (10), 109 (10).
4b	2928, 2855, 1602, 1488, 1457, 1262	1.19–2.26 (m, 10H, 5 CH_2), 6.96–7.10 (m, 2H, ArH), 7.26–7.44 (m, 2H, ArH), 7.57–7.72 (m, 2H, ArH), 7.86 (s, 1H, ArH), 8.05 (d, *J* = 8.6 Hz, 1H, ArH), 8.39–8.44 (m, 1H, ArH).	301 (M, 26), 300 (M −H, 31), 272 (11), 257 (100), 245 (14), 216 (17), 188 (5).
4c	2969, 2937, 1602, 1490, 1457, 1241	0.84–0.91 (m, 3H, CH_3CH_2), 1.68 (s, 3H, CH_3), 1.75–1.89 (m, 1H, C*Ha*HbCH$_3$), 1.96-2.14 (m, 1H, CHa*Hb*CH$_3$), 6.90 (d, *J* = 8.0 Hz, 1H, ArH), 7.00–7.07 (m, 1H, ArH), 7.17–7.44 (m, 2H, ArH), 7.57–7.78 (m, 2H, ArH), 8.04 (d, *J* = 8.4 Hz, 1H, ArH), 8.41 (d, *J* = 7.6 Hz, 1H, ArH), 7.78 (s, 1H, ArH).	275 (M, 10), 260 (M − CH_3, 6), 246 (M − C_2H_5, 100), 217 (13), 123 (8).
4d	2963, 1446, 1261	1.77 (s, 6H, 2 CH_3), 6.99 (d, *J* = 8.1 Hz, 1H, ArH), 7.14 (t, *J* = 7.5 Hz, 1H, ArH), 7.35–7.38 (m, 1H, ArH), 7.48–7.51 (m, 1H, ArH), 7.67–7.76 (m, 1H, ArH), 7.86 (s, 1H, ArH), 8.09–8.14 (m, 1H, ArH), 7.47 (d, *J* = 7.8 Hz, 1H, ArH).	279 (M, 23), 264 (M − CH_3, 100), 235 (12), 149 (2).
4e	2928, 2858, 1608, 1494, 1457, 1298, 1262	1.18–1.90 (m, 8H, 4 CH_2), 2.21 (d, *J* = 14.0 Hz, 2H, CH_2), 6.95–7.09 (m, 2H, ArH), 7.18 (s, 1H, ArH), 7.25–7.42 (m, 2H, ArH), 7.80 (s, 1H, ArH), 7.99–8.06 (m, 1H, ArH), 8.36 (dd, *J* = 7.8, 1.8 Hz, 1H, ArH)/ 21.41, 25.28, 35.17, 78.43, 110.68 (d, $J_{C\text{-}F}$ = 21.75 Hz), 117.87, 119.46 (d, $J_{C\text{-}F}$ = 25.50 Hz), 121.95, 123.04, 125.26, 128.38 (d, $J_{C\text{-}F}$ = 10.50 Hz), 129.01, 131.59 (d, $J_{C\text{-}F}$ = 9.00 Hz), 131.68, 134.80, 144.60, 147.76, 154.71, 160.23 (d, $J_{C\text{-}F}$ = 246.00 Hz).	319 (M, 34), 275 (M − C_3H_8, 100), 262 (13), 234 (11).
4f	3047, 2971, 2925, 2876, 1601, 1498,1460, 1215	0.85-0.90 (m, 3H, CH_3CH_2), 1.70 (s, 3H, CH_3), 1.76–1.89 (m, 1H, C*Ha*HbCH$_3$), 2.01–2.11 (m, 1H, CHa*Hb*CH$_3$), 7.00 (d, *J* = 8.1 Hz, 1H, ArH), 7.12 (t, *J* = 7.4 Hz, 1H, ArH), 7.38–7.43 (m, 1H, ArH), 7.63–7.78 (m, 2H, ArH), 8.09 (d, *J* = 8.1 Hz, 1H, ArH), 8.30–8.34 (m, 2H, ArH).	293 (M, 23), 264 (M − C_2H_5, 100), 235 (11), 132 (7).
4g	3745, 2963, 2923, 1492, 1460, 1252	1.78 (s, 6H, 2 CH_3), 7.00 (d, *J* = 8.1 Hz, 1H, ArH), 7.14–7.18 (m, 2H, ArH), 7.30 (d, *J* = 8.4 Hz, 1H, ArH), 7.36–7.40 (m, 2H, ArH), 7.93 (s, 1H, ArH), 8.2 (bs, 1H, OH exchangeable with D_2O), 8.43 (d, *J* = 7.8 Hz, 1H, ArH).	277 (M, 37), 262 (M − CH_3, 100), 233 (7), 117 (9).
4h	3445, 2924, 2854, 2364, 1629, 1543, 1485, 1454, 1254	0.89-1.81 (m, 10H, 5 CH_2), 6.99–7.05 (m, 1H, ArH), 7.12–7.18 (m, 1H, ArH), 7.38–7.47 (m, 3H, ArH), 7.60–7.63 (m, 1H, ArH), 7.72–7.76 (m, 1H, ArH), 8.04 (dd, *J* = 7.8, 1.2 Hz, 1H, ArH), 11.42 (bs, 1H, OH exchangeable with D_2O).	273 (M − C_3H_8, 70), 154 (56), 96 (40), 55 (100).

Table 2. Continued

Compd.	IR (cm^{-1})	^1H- and ^{13}C-NMR (δ ppm)	MS m/z (%)
4i	3368, 2971, 2925, 2876, 1602, 1491,1463	0.86 (t, *J* = 7.4 Hz, 3H, CH_3CH_2), 1.70 (s, 3H, CH_3), 1.82–1.84 (m, 1H, C*Ha*HbCH_3), 2.06–2.15 (m, 1H, CHa*Hb*CH_3), 6.96 (d, *J* = 8.5 Hz, 1H, ArH), 7.01–7.15 (m, 2H, ArH), 7.37–7.39 (m, 3H, ArH), 8.20 (s, 1H, ArH), 8.73 (d, *J* = 8.1 Hz, 1H, ArH), 9.63 (bs, 1H, OH, exchangeable with D_2O).	291 (M, 21), 262 (M − C_2H_5, 100), 233 (8), 204 (5), 131 (4).
5a	2924, 1561, 1454, 1252	1.73 (s, 6H, 2 CH_3), 7.11–7.19 (m, 2H, ArH), 7.36–7.42 (m, 1H, ArH), 7.57–7.62 (m, 1H, ArH), 7.69–7.75 (m, 1H, ArH), 8.04 (dd, *J* = 7.8 Hz, 1.5 Hz, 1H, ArH), 8.13–8.16 (m, 1H, ArH), 8.53–8.56 (m, 1H, ArH), 8.33 (s, 1H, CH=N).	261 (M, 14), 246 (M −CH_3, 100), 217(7), 189 (5).
5b	2930, 2854, 1558, 1501, 1444, 1245	1.62–1.99 (m, 10H, 5 CH_2), 7.20–7.25 (m, 2H, ArH), 7.43-7.46 (m, 1H, ArH), 7.68—7.71 (m, 1H, ArH), 7.78 (dd, *J* = 8.4, 1.5 Hz, 1H, ArH), 8.05–8.08 (m, 2H, ArH), 8.51 (d, *J* = 8.7 Hz, 1H, ArH), 8.97 (s, 1H, CH=N).	301 (M, 30), 300 (M −H, 4), 272 (10), 258 (100), 245 (14), 216 (6), 189 (10).
5c	2971, 2930, 1604, 1483, 1454, 1253	0.83–0.88 (m, 3H, CH_3CH_2), 1.66 (s, 3H, CH_3), 1.80–1.88 (m, 1H, C*Ha*HbCH_3), 1.97–2.03 (m, 1H, CHa*Hb*CH_3), 7.11–7.22 (m, 2H, ArH), 7.40–7.46 (m, 1H, ArH), 7.67 (t, *J* = 7.8 Hz, 1H, ArH), 7.75–7.80 (m, 1H, ArH), 8.04–8.09 (m, 2H, ArH), 8.51 (d, *J* = 8.4 Hz, 1H, ArH), 8.89 (s, 1H, CH=N).	275 (M, 6), 246 (M − C_2H_5, 100), 217 (6), 123 (4).
7	2925, 2644, 1591, 1548, 1442, 1286, 756	1.61–1.85 (m, 8H, 4 CH_2), 2.50 (d, *J* = 12.0 Hz, 2H, CH_2), 4.71 (s, 1H, SH, exchangeable with D_2O), 6.92–7.32 (m, 6H, ArH), 7.60–7.65 (m, 2H, ArH), 8.82 (s, 1H, CH=N).	369 (M, 56), 333 (M − HCl, 85), 299 (56), 276 (42), 258 (100), 136 (57), 109 (30), 91 (18).

(Table 2). The IR spectrum of **4g** shows OH at *v* 3745 cm^{-1}. The ^1H-NMR spectrum of **4g** shows OH at δ 8.2 as a singlet, which is exchangeable with D_2O. The mass spectrum of **4g** shows the prominent ion peak M^+ at m/z 277 (37%).

Simay and Takács have reported the synthesis of the fused heterocycles pyrazolo[4′,3′:5,6]pyrido[2,3-*b*][1,5]benzothiazepine and pyrazolo[4′,3′:5,6]pyrido[2,3-*b*][1,5]benzoxazepine [27]. In the present work, surprisingly, 4-chloro-2,2-disubstituted(2*H*)chromene-3-carboxyaldehydes **3a–c** reacted with 2-aminothiophenol in acetic acid under reflux for 3 h and afforded (6*H*)chromeno[3,4-*c*]quinolines **5a–c** through the corresponding 1,4-thiazepine **6** after extrusion of sulfur (Scheme 1). The structures of **5a–c** were established by physical and spectral data (IR, ^1H-NMR, and MS) (Tables 1, 2) as well as elemental analyses. So, the IR spectrum of **5b** shows HC=N at *v* 1558 cm^{-1}, its ^1H-NMR shows CH=N at δ 8.97 as a singlet. The mass spectrum of **5b** shows the prominent ion peak M^+ at *m/z* 301 (30%). Moreover, the single crystal X-ray determination of **5b** gives a good evidence for the products (Fig. 2).

4-Chlorospiro(2*H*)chromene(2,1′)cyclohexane-3-carboxyaldehydes **3b** reacted with 2-aminothiophenol in ethanol under reflux for 30 min to give 4-chloro-3-(2-thiolphenyl)iminomethylene spiro(2*H*)chromene(2,1′)cyclohex-

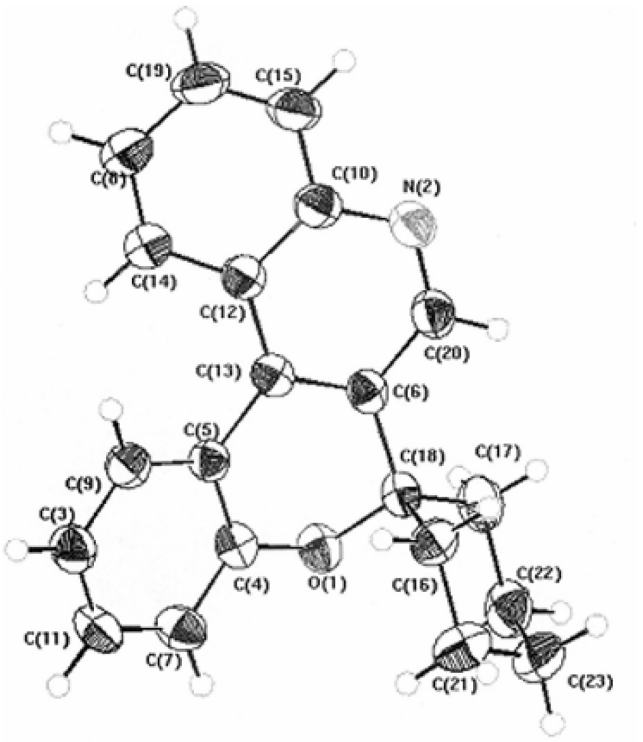
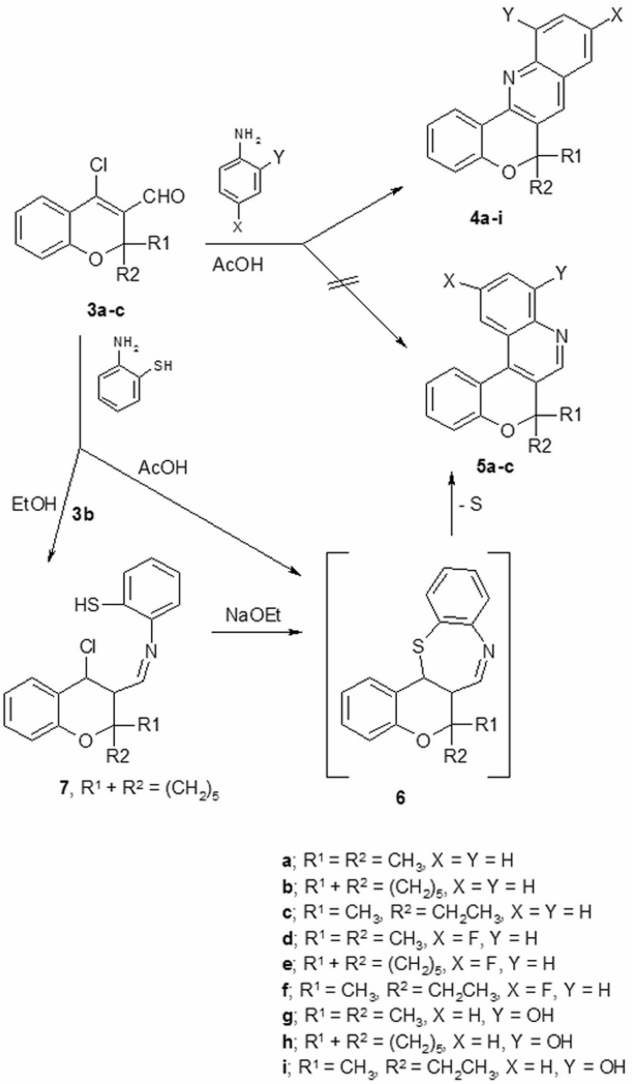


Figure 2. Molecular structure of compound **5b;** ellipsoids of thermal vibration are shown with 50% probability.



Scheme 2. Synthesis route of compounds **4** and **5.**

ane **7**, which was treated with ethanolic sodium ethoxide to obtain **5b** (Scheme 1). The structure of **7** was established by physical and spectral data (Tables 1, 2) as well as elemental analyses. The IR spectrum of **7** shows (SH) at ν 2644 cm^{-1} and (C=N) at 1591 cm^{-1}. ^{1}H-NMR spectrum of **7** shows SH at δ 4.71 (exchangeable with D_2O), CH=N at δ 8.82. Its MS shows the prominent ion peak at 369 (M^+, 56). The formation of 6,6-disubstituted chromeno[4,3-*b*]quinolines **4a–i** can be explained via the following Scheme 2.

Pharmacological screening

Eight representative compounds **4a–c**, **4f**, **4i**, **5a–c** were studied with respect to anti-inflammatory and ulcerogenic activities.

Table 3. Anti-inflammatory activity for derivatives **4a–c, 4f, 4i, 5a,b**, and **5c.**

Compound	Anti-inflammatory activity (5mg/kg, p.o) (inhibition ± S.E.M) Ulcerogenic score		
	1 h	2 h	3 h
Indomethacin	43 ± 5	56 ± 6	62 ± 5
4a	17 ± 1	27 ± 2	33 ± 2
4b	24 ± 2	35 ± 2	40 ± 1
4c	21 ± 5	31 ± 5	35 ± 4
4f	15 ± 6	25 ± 6	31 ± 5
4i	13 ± 5	21 ± 4	25 ± 5
5a	31 ± 2	40 ± 2	45 ± 3
5b	42 ± 2	51 ± 1	56 ± 2
5c	38 ± 3	49 ± 3	54 ± 2

Significant difference at $p < 0.05$.

Anti-inflammatory activity

The anti-inflammatory activities of the tested compounds were evaluated by carrageenan-induced paw edema by the method of Hernandez–Perez *et al*. [36]. The compounds were tested at 5mg/kg oral dose and were compared with indomethacin as reference drug. The results are listed in Table 3. The histogram (Fig. 1) showed the percent inhibition of edema induced by the reference drug and tested compounds, respectively. Results showed that three compounds, **4b, 5b**, and **5c** of the tested compounds showed significant ($p < 0.05$) inhibition against carrageenan-induced edema in rats.

The anti-inflammatory activities of the tested compounds ranged from 25–55%, whereas the standard drug, indomethacin showed an activity of 62% after 3 h. The anti-inflammatory activity of chromenoquinoline derivatives **4a–c, 4f, 4i, 5a,b**, and **5c** revealed that 6-ethyl-6-methyl-11-hydroxy(6*H*)chromeno[4,3-*b*]quinoline **4i** was found to be of lower activity (25%) than the other tested compounds (28–55%). The regioisomer chromeno[4,3-*b*]quinolines **4a–c** have lower activity (33–40%) than the other regioisomer chromeno[3,4-*c*]quinolines **5a–c** (45–56%). On the other hand, derivatives **5c** and **5b** showed maximum inhibition of inflammation 54 and 56%, respectively. The inhibition (in %) of carrageenan-induced edema by indomethacin and the test compounds is shown in Fig. 3 for **4a–c, 4f** and **4i** and in Fig. 4 for **5a–c**.

Ulcerogenic activity

The tested compounds **4a–c, 4f, 4i, 5a,b** and **5c** were screened for their ulcerogenic activity at dose level 10, 50, 100 mg/kg (Table 4; [37]). The tested compounds **5b** and **5c** have no ulcerogenic toxicity, while **4a–c, 4f**, and

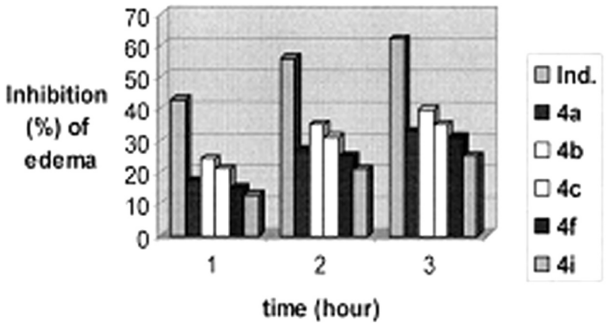


Figure 3. Inhibition (%) of carrageenan-induced edema by indomethacin (Ind.) and test compounds **4a–c, 4f** and **4i.**

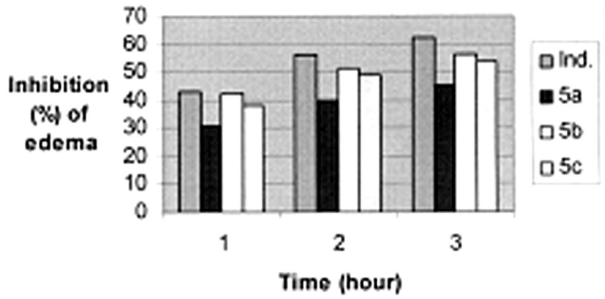


Figure 4. Inhibition (%) of carrageenan-induced edema by indomethacin (Ind.) and test compounds **5a–c.**

Table 4. Gastric ulceration in rats[a].

Compound	Dose (mg/kg)		
	10	**50**	**100**
Indomethacin	3/6 (1.4 ± 0.18)[b]	5/6 (1.9 ± 0.15)[b]	6/6 (2.1 ± 0.17)[b]
4a	0/6 (0.00)	0/6 (0.00)	2/6 (1.2 ± 0.08)[b]
4b	0/6 (0.00)	0/6 (0.00)	2/6 (1.1 ± 0.07)[b]
4c	0/6 (0.00)	0/6 (0.00)	2/6 (1.1 ± 0.07)[b]
4f	0/6 (0.00)	0/6 (0.00)	3/6 (1.65 ± 0.09)[b]
4i	0/6 (0.00)	0/6 (0.00)	3/6 (1.75 ± 0.10)[b]
5a	0/6 (0.00)	0/6 (0.00)	1/6 (0.75 ± 0.06)[b]
5b	0/6 (0.00)	0/6 (0.00)	0/6 (0.00)
5c	0/6 (0.00)	0/6 (0.00)	0/6 (0.00)

[a] Number of rats with lesions more than 0 mm in length per total number of rats. The number in parenthese is the mean ulcer index [mm] with S.E.M.

[b] Significant difference at $p < 0.05$.

4i showed mild ulceration at a dose of 100 mg/kg. Compound **5a** showed lower ulcerogenic activity at 100 mg/kg (0.75 ± 0.06), compared with indomethacine which showed ulcerogenic activity from (1.35 ± 0.18 to 2.1 ± 0.17).

Acute toxicity (LD_{50})

LD_{50} of the most active compounds **5b** and **5c** was determined using a graphical method [38]. LD_{50} of compounds **5b** and **5c** was found to be 150 mg/kg and 165 mg/kg (i.p.), respectively, while LD_{50} of indomethacin is 50 mg/kg (i.p.).

Experimental

Melting points were determined on open glass capillaries using Electrothermal IA 9000 SERIES digital melting point apparatus (Electrothermal, Essex, U.K.) and are uncorrected. Microanalyses were performed with all final compounds on Elementar-Vario EL, Microanalytical Unit, Central Services Laboratory, National Research Centre, Cairo, Egypt and were found within ± 0.3% of the theoretical values. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA). ^{1}H-NMR spectra were run at 300 MHz and ^{13}C-NMR spectra were run at 75.46 MHz in $CDCl_3$ as solvent. Chemical shifts are quoted in δ and were related to that of the solvents (Micro-analytical Center of Cairo University, Cairo). Splitting patterns were given as follow: s: singlet; d: doublet; t: triplet; m: multiplet. Mass spectra were recorded on Shimadzu GCMS-QP 1000EX (EI, 70 eV; Shimadzu, Tokyo, Japan), (Micro-analytical Center of Cairo University, Cairo). IR spectra were obtained with Brucker-Vector 22 (Bruker Bioscience, Billerica, MA, USA) for neat samples (for liquids) or KBr wafers (for solid) (Micro-analytical Center of Cairo University, Cairo). Compounds **1a, c** [28], **1b** [29], **2a** [30], **2b** [31], **3a, b** [6] were prepared according to the literature.

Chemistry

*Reaction of ketones **1c** with Vilsmeier reagent*

In a three-necked flask, was placed dimethylformamide (2.8 g, 37 mmol) and methylene chloride (20 mL). After cooling in an ice bath to 0 – 5°C, phosphorus oxychloride (2.7 g, 30 mmol) was added dropwise. Addition was regulated to maintain the temperature of the mixture below 20°C. After the addition of phosphorus oxychloride was completed, the reaction was continued at room temperature for 2 h. Following this period, the mixture was cooled again in an ice bath and a solution of ketone **1c** (3.5 g, 18 mmol) in methylene chloride (30 mL) was added dropwise to the mixture while maintaining the temperature of the reaction below 20°C with external cooling. After this addition was completed, the reaction was continued at room temperature for 24 h and then poured over crushed ice; solid sodium bicarbonate was added until the formation of carbon dioxide ceased. After the mixture had been stirred at room temperature for 2 h, the aqueous layer was extracted with methylene chloride. The aqueous portion was extracted twice with 80 mL of methylene chloride. The combined methylene chloride extracts were washed twice with water (50 mL), dried with sodium sulfate, and evaporated under reduced pressure. Chromatography of the residue over silica gel (Merck 60, particle size 0.06 – 0.20 mm, Merck, Darmstadt, Germany), petroleum ether 60 – 80°C was used as an eluent to afford **2c** and **3c**.

*Reaction of β-chloro carboxyaldehyde **3a, b, c** with aniline, fluoroaniline and 2-aminophenol*

An appropriate aniline derivatives (1.04 mmol) was poured into a solution of the β-chloro carboxyaldehyde **3a, 3b**, or **3c** (1.04 mmol) in acetic acid (15 mL). The reaction mixture was

refluxed for 3 h and then evaporated under reduced pressure. The residue was dissolved in dichloromethane and the resulting solution was washed with 5% aqueous $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated to dryness. Chromatography of the residue over silica gel (Merck 60, particle size 0.06–0.20 mm; Merck), petroleum ether 60–80°C : diethyl ether (40 : 1) was used as an eluent to afford the corresponding products **4a–i**.

*Reaction of β-chloro carboxyaldehyde **3a, b, c** with 2-aminothiophenol in acetic acid*

2-Aminothiophenol (0.80 g, 2.7 mmol) was poured into a solution of the β-chloro carboxyaldehyde **3a**, **3b**, or **3c** (2.7 mmol) in acetic acid (15 mL). The reaction mixture was refluxed for 3 h and then evaporated under reduced pressure. The residue was dissolved in dichloromethane and the resulting solution was washed with 5% aqueous $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated to dryness. Chromatography of the residue over silica gel (Merck 60, particle size 0.06–0.20 mm), petroleum ether 60–80°C : diethyl ether (40 : 1) was used as an eluent to afford the corresponding products **5a–c**.

*Reaction of β-chloro carboxyaldehyde **3b** with 2-aminothiophenol in ethanol*

2-Aminothiophenol (0.19 g, 1.58 mmol) was poured into a solution of β-chloro carboxyaldehyde **3b** (0.41 g, 1.56 mmol) in ethanol (15 mL). The reaction mixture was refluxed for 30 min. The formed solid was filtered off hot to afford a pure 4-chloro-3-(2-thiolphenyl)iminomethylene spiro(2*H*)chromene(2,1′)-cyclohexane **7**.

*Reaction of compound **7** with ethanolic sodium ethoxide*

To an ethanolic sodium ethoxide solution prepared from sodium (0.02 g, 1 mmol) and absolute ethanol (15 mL) **7** (0.36 g, 1 mmol) was added. The mixture was refluxed for 5 h, and then unreacted compound **7** was filtered off. The filtrate was evaporated under reduced pressure and the residue was purified by column chromatography (silica gel 0.06–0.2 particle size, $CHCl_3$ as an eluent) to afford 0.28 g (93%) **5b**.

X-Ray data collection

A suitable crystal of compounds **4e** and **5b** was selected and mounted with epoxy on the tip of a fine glass fiber. All X-ray crystallographic measurements were made at 298 K on an Enraf-Nonius 590 Kappa CCD single crystal diffractometer equipped with graphite monochromatized Mo-Ka radiation (λ = 0.71073 Å) operating in φ-ω scan mode; the crystal to detector distance was 40 mm. Further details are summarized in Table 1. The cell refinement and data reduction were carried out using Denzo and Scalepak programs [32], the structures were solved by the direct method using the SIR92 program [33] and anisotropic displacement parameters were applied to non-hydrogen atoms in full-matrix least-squares refinement based on F^2 using the maXus package [34]. Then, the hydrogen atoms bonded to the carbon were included using the riding model. The final cycle of the full-matrix least-squares refinement was based on 1960 observed reflections with I > 3s (I) and 433 variable parameter for the compound **4e** and 1629 reflections with I > 3s (I) and 208 variable parameter for the compound **5b** The molecular graphics were prepared using the ORTEP program [35].

Pharmacology

Materials and methods

Materials: Indomethacin was purchased from Nile Co. (Nile Co., Cairo, Egypt). Carrageenan, used to induce edema, was purchased from Sigma Chemicals Co., St. Louis, MO, USA. Plethysmometer 7150, (Ugo Basile, Italy) was used to measure the volume of paw edema.

Animals: Both mice and rats used were Wister albino of either sex, produced from National Research Centre, Giza, Egypt; they were housed under suitable laboratory conditions through the period of investigation. Animals were fed standard pellet chow (El-Nasr Chemical Company, Cairo, Egypt) and allowed free access to water. The data for activity and toxicity were evaluated statistically using Student's *t*-test. A level of $p < 0.05$ was adopted for the test of significance.

Anti-inflammatory activity

Anti-inflammatory activity of the compounds under investigation was studied in rats using carrageenan. A suspension of the tested compounds and reference drug indomethacin in carboxy methylcellulose (CMC) solution (0.5% w/v in water) was administrated orally to rats in one dose (5 mg/kg). Control animals were treated similarly with CMC (0.5% w/v in water).

After 30 min, 0.1 mL of freshly prepared 1% carrageenan solution in normal saline was injected into the subplantar region of the right hind paw according to the method of Hernandez–Perez *et al*. [36]. The right paw volume was measured by Plethysmometer 7150, directly before and at 1, 2, 3 h intervals after administration of the tested compounds. The anti-inflammatory activity of the tested compounds and reference drug was determined with the following formula [39]:

$$\% \text{ Anti-inflammatory activity} = (Vc - Vt/Vc) \times 100$$

Where Vc represents the mean increase in paw volume in the control group of rats. Vt represents the mean increase in paw volume in rats treated with tested compounds and data are expressed as mean ± S.E.M., the students *t*-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds.

Ulcerogenic activity [37]

Albino rats have been divided into different groups containing six animals in each group. Ulcerogenic activity was evaluated after p.o. administration of the tested compounds or indomethacin at doses of 10, 50, and 100 mg/kg, control rats received the vehicle p.o (suspension of 0.5% w/v CMC). Food but not water was removed 24 h before administration of the tested compounds. After 6 h, the rats were scarified and the stomach was removed, and opened along the greater curvature, washed with distilled water and cleaned gently by dipping into saline. The mucosed damage for each stomach was examined using a stereoscopic microscope, the mucosal damage was compared with indomethacin. The mean score of each treated group was regarded as severity index of gastric mucosal damage. Data are expresses as mean ± S.E.M., the Students *t*-test was applied to determine the significance of the difference between the standard group and rats treated with the tested compounds.

Acute toxicity (LD_{50}) [38]

The median lethal doses (LD_{50}) of the most active compounds **5c** and **5b** were determined in mice. Groups of male adult albino mice, each consisting of six animals, were injected i.p. with graded doses of each of the test compounds. The percentage of mortality in each group of animals was determined 24 h after injection. Computation of LD_{50} was processed by a graphical method.

References

[1] B. C. Sekhar, S. R. Ramadas, D. V. Ramana, *Heterocycles* **2000**, *53*, 941 – 977.

[2] T. Aubert, M. Farnier, I. Meunier, R. Guilard, *J. Chem. Soc. Perkin Trans. I* **1989**, 2095 – 2098.

[3] M. I. El-Deen, *Chin. J. Chem.* **1999**, *17*, 391 – 397.

[4] M. I. El-Deen, I. K. H. Abd El – Fattah, A. El – Gohary, *Phosphorus Sulfur Silicon Relat. Elem.* **2003**, *178*, 1463 – 1476.

[5] B. K. Clarke, D. N. Gregory, R. M. Scrowston, *J. Chem. Soc. Perkin Trans. I* **1973**, 2956 – 2960.

[6] M. I. Hegab, M. M. Abdulla, *Arch. Pharm. Chem. Life Sci.* **2006**, *339*, 41 – 47.

[7] D. Pan, G. K. Kar, J. K. Ray, J. M. Lin, *et al.*, *J. Chem. Soc. Perkin Trans. I* **2001**, 2470 – 2475.

[8] T. Sugaya, Y. Mimura, Y. Shida, Y. Osawa, *et al.*, *J. Med. Chem.* **1994**, *37,* 1028 – 1032.

[9] M. S. Sham, B. Gurudas, K. J. Rafid, S. Rakesh, *et al.*, *Cent. Eur. J. Chem.* **2004**, *2*, 1 – 15.

[10] V. K. Misra, S. C. Bahel, *J. Ind. Chem. Soc.* **1984**, *61*, 916 – 918.

[11] A. Srivastava, R. B. Pathak, S. C. Bahel, *J. Ind. Chem. Soc.* **1985**, *62*, 486 – 487.

[12] L. Nagadi, N. G. Bisri, A. Mahmoud, A. M. Galy, *et al.*, *Arz. Forsch.* **1993**, *43*, 480 – 483.

[13] I. S. Shulga, A. K. Sukhomlinov, A. I. Goncharov, E. M. Dikaya, *Farm. Zh. (Kiev)* **1974**, *29*, 27 – 29. *Chem. Abstr.* **1974**, *81*, 105236s.

[14] N. A. Gaiukevich, G. S. Bashura, I. M. Pestsev, O. A. Pimenov, A. I. Pyatkop, *Farm. Zh. (Kiev)* **1973**, *28*, 50 – 54.

[15] S. G. Isaev, I. S. Shulga, A. V. Kaliman, N. E. Sheveleva, L. F. Silaeva, *Farm. Zh. (Kiev)* **1988**, *2*, 68 – 69. *Chem. Abstr.* **1988**, *109*, 31552e.

[16] I. B. Taraporewala, J. M. Kauffman, *J. Pharm. Sci.* **1990**, *79*, 173 – 178.

[17] A. N. Gaidukevich, G. P. Kazakov, A. A. Kravchenko, L. A. Porokhnyak, V. V. Dinchuk, *Khim. Farm. Zh.* **1987**, *21*, 1067 – 1070; *Chem. Abstr.* **1988**, *108*, 68486s.

[18] S. M. Sondhi, G. Bhattacharjee, R. K. Jameel, R. Shukla, *et al.*, *Cent. Europ. J. Chem.* **2004**, *2*, 1 – 15.

[19] K. H. Mekouar, J. F. Mouscadet, D. Desmaele, F. Subra, *et al.*, *J. Med. Chem.* **1998**, *41*, 2846 – 2857.

[20] F. Zouhiri, M. Danet, C. Bénard, M. Normand-Bayle, *et al.*, *Tetrahedron Lett.* **2005**, *46*, 2201 – 2205.

[21] Y. Pommier, A. A. Johnson, C. Marchand, *Nat. Rev. Drug. Discov.* **2005**, *4*, 236 – 248.

[22] R. Musiol, J. Jampilek, K. Kralova, B. Podeszwa, *et al.*, 9th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-9), 1 – 30 November **2005**, http//www.usc.es/congresos/ecsoc/9/ecsoc9.HTM & http//www.mdpi.net/ecsoc/.

[23] M. A. Pomce, J. M. Scervino, R. Erra-Balsells, J. A. Occampo, A. M. Godeas, *Phytochemistry* **2004**, *65*, 3131 – 3134.

[24] F. Cottiglia, B. Dhanapal, O. Sticher, J. Heilmann, *J. Nat. Prod.* **2004**, *67*, 537 – 541.

[25] J. Mutanyatta, B. Matapa, D. D. Shushu, B. M. Abegaz, *Phytochemistry* **2003**, *62*, 797 – 804.

[26] Y. Lin, Y. Zhou, M. T. Flavin, L. Zhou, *et al.*, *Bioorg. Med. Chem.* **2002**, *10*, 2795 – 2802.

[27] A. Simay, K. Takács, *J. Heterocyclic Chem.* **1982**, *19*, 809 – 811.

[28] F. A. G. El-Essawy, S. M. Yassin, I. A. El – Sakka, A. F. Khattab, *et al.*, *J. Org. Chem.* **1998**, *63*, 9840 – 9845.

[29] H. J. Kabbe, *Synthesis* **1978**, 886 – 887.

[30] A. Alberola, B. Calvo, A. G. Ortega, R. Pedrosa, *J. Chem. Soc. Perkin Trans. I* **1992**, 3075 – 3080.

[31] T. Eszenyi, T. Tímár, P. Sebók, *Tetrahedron Lett.* **1991**, *32*, 827 – 828.

[32] Z. Otwinowski, W. Minor in *Processing of X-ray Diffraction Data Collected in Oscilation Mode*, Methods in Enzymology, Vol. 276: Macromolecular Crystallography Part A, (Eds.: C.W. Carter, Jr. & R. M. Sweet), Academic Press, New York, **1997**.

[33] A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, *et al.*, *J. Appl. Cryst.* **1999**, *32*, 115 – 119.

[34] S. Mackay, C. J. Gilmore, C. Edwards, N. Stewart, K. Shankland, *maXus Computer Program for the Solution and Refinement of Crystal Structures* Bruker Nonius, The Netherlands, MacScience, Japan & The University of Glasgow, **1999**.

[35] C. K. Johnson, *ORTEP-II. A Fortran Thermal-Ellipsoid Plot Program. Report ORNL-5138* Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA, **1976**.

[36] M. Hernandez-Perez, R. M. Rabanal, M. C. de la Torre, B. Rodriguez, *Planta Med.* **1995**, *61*, 505 – 509.

[37] H. Ikuta, H. Shirota, S. Kobayashi, Y. Yamagishi, *et al.*, *J. Med. Chem.* **1987**, *30*, 1995 – 1998.

[38] F. Sztaricskai, I. E. Takaccs, F. Pusztai, G. Szabo, I. Cssipo, *Arch. Pharm. Med. Chem.* **1999**, *332*, 321 – 326.

[39] H. Nojima, H. Tsuneki, I. Kimura, M. Kimura, *Br. J. Pharmacol.* **1995**, *116*, 1680 – 1684.

